

Sex Differences in Total and Specific Antioxidant Enzymes in Unirradiated and
Irradiated Skin

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Abstract

Nonmelanoma skin cancers have been reported to be more predominant in the male gender with the incidence of basal cell carcinoma and squamous cell carcinoma in males being two and three times the incidence in females respectively. Until recently these differences have been attributed to lifestyle variations amongst men and women in which men seem to spend more time outside while using less sun protection. Our laboratory and others have established a correlation between UVB-induced cutaneous inflammation and tumor susceptibility in the skin of female mice. These findings were compared to male mice in a recent study done by Thomas-Ahner et. al. (Cancer Research 2007) in our lab to evaluate the gender differences in UVB-induced skin carcinogenesis, inflammation, and DNA damage. Using the Skh-1 mouse model, Thomas-Ahner found that under equal doses of UVB exposure, male mice tend to have an accelerated tumor progression, less of an inflammatory response, more cutaneous oxidative DNA damage, and lower antioxidant levels compared to the female mice. The goal of the current study was to continue the ongoing investigation into the gender disparities by examining the temporal and gender differences in UVB-induced inflammation and the total and specific antioxidant enzymes, including catalase, glutathione peroxidase, glutathione s-transferase, and glutathione reductase, after a single exposure to UVB. We found that UV-induced cutaneous inflammation increased in both genders but to a greater extent in females. We also determined that catalase was lower at baseline in male skin and decreased equally in both genders after UV; there were no significant gender or UV mediated differences in superoxide dismutase or glutathione reductase; glutathione peroxidase and glutathione s-transferase were lower at baseline in male skin and

glutathione s-transferase decreased in the skin of both genders after UV exposure while glutathione peroxidase only decreased in males after UV exposure. These results demonstrate that there are specific inherent differences in the skin of the genders. Furthermore these studies suggest that males may not be able to ward off cutaneous DNA damage as well as females due to the lower antioxidant activities found in male skin in both unirradiated skin and after UV exposure. Taken together, the results from our current and our published studies suggest that there is a possible biological explanation for the higher levels of skin cancer in men.

Introduction

The American Cancer Society reports that skin cancer, which includes melanoma and squamous and basal cell skin carcinomas, is the most predominant form of all cancers counting for about half of all cancer cases. Over 1 million new cases of basal and squamous cell skin cancers alone are estimated to be diagnosed every year in the United States. It has been reported that nonmelanoma skin cancers are more predominant in the male gender with the incidence of basal cell carcinoma and squamous cell carcinoma in males being two and three times the incidence in females respectively (1). These differences have been attributed to lifestyle variations amongst men and women in which men seem to spend more time outside while using less sun protection (2,3). This is an area of interest to our laboratory to determine if there are inherent biological differences that lead to this gender disparity in the incidence of skin cancer.

It has been well established that chronic exposure to UV radiation promotes the development of nonmelanoma skin cancer (reviewed in ref. 4). The UVB portion of the UV spectrum has been proven to be responsible for the most UV-induced carcinogenicity (reviewed in ref. 5). The initial effect of UV irradiation on the skin is an acute inflammatory response. This cutaneous inflammatory response is characterized by redness (erythema) and swelling (edema) of the skin which leads to the activation and infiltration of inflammatory cells including neutrophils and macrophages (reviewed in refs. 4,5,6). There is a substantial amount of evidence supporting a clear link between inflammation and the development and progression of many types of cancer. Our laboratory and others have demonstrated a clear relationship between inflammation and skin cancer. Topical application of the anti-inflammatory drug Celecoxib inhibited the

acute inflammatory response after a single UVB exposure and decreased tumor formation after chronic exposure to UVB in female mice. This study showed that there appeared to be a direct correlation between inflammation and the development of tumors after UV exposure in female mice (8). It had not, however, been determined whether this same relationship existed in male mice.

There have been several reports of gender differences in the development of various diseases. Our laboratory, however, was the first known laboratory to conduct a controlled murine study in 2007 which examined the gender differences in UVB induced skin carcinogenesis, inflammation, and DNA damage to determine if males have a similar response to UVB as females. This was the first study to demonstrate that under equal doses of UVB exposure, male mice have an increased tumor burden, decreased tumor latency, and tumors of higher grade than the tumors of female mice (9). This study supports the epidemiological reports that men have a higher incidence of skin cancer but refutes the belief that lifestyle is the major culprit for the gender disparities (1,2,3). Ironically this study also found that male mice had less of an inflammatory response compared to that of female mice (9). Based on the anti-inflammatory effects of Celecoxib after UVB exposure and the decreased tumor formation found in previous studies (8), male mice were expected to have a greater inflammatory response if they displayed a greater tumor burden than female mice which did not prove to be true. Given that the difference in the inflammatory response to UVB did not correlate as expected with the difference in skin tumor susceptibility between the male and female mice, the biological basis for gender differences in skin carcinogenesis may be found in the oxidative repair mechanisms for each gender (9).

Ultraviolet radiation can cause direct or indirect biological damage which can lead to the development of skin carcinogenesis. UV radiation can cause DNA damage directly through the formation of cyclobutane pyrimidine dimers (CPD) or indirect DNA damage through the production of ROS by activated inflammatory cells (9, reviewed in refs. 6,10). Reactive oxygen species, which include superoxide anion (O_2^-), hydroxyl radical ($^{\bullet}OH$), and hydrogen peroxide (H_2O_2), are oxygen-based molecules that are very reactive with an unpaired electron in their outermost orbit. These ROS are generated under normal cellular functions but usually cause minimal harm because of intracellular mechanisms that dissipate their damaging effects (reviewed in refs. 11,12). ROS may also, however, be derived from many exogenous sources such as ultraviolet radiation. Prolonged free radical activity through overproduction or deficient eradication of ROS can lead to oxidative stress which may result in deleterious effects on the skin. Such deleterious effects to skin cells include dysregulation of the cell cycle, disruption in the DNA repair and/or replication process, direct DNA damage, and gene mutations (reviewed in ref. 12). One common marker of DNA damage caused by oxidative stress is the formation of 8-hydroxydeoxyguanosine (8-OHdG) in the DNA. The DNA is mutated through the transfer of an electron from a ROS produced by UV that triggers a DNA base change in guanine which is the DNA base most sensitive to oxidation (reviewed in refs. 6,13). The 8-OHdG by product appears not only in mice after UV exposure but in normal human epidermis following UV irradiation (reviewed in ref. 12).

There are several intracellular antioxidants and antioxidant enzymes that work to delay or prevent cellular damage caused by reactive oxygen species. The antioxidants function by dissipating the detrimental effects of O_2 in the lipid and nonlipid portions of

the cell. Cellular antioxidant enzymes regulate the actions of ROS or their byproducts through various intracellular chemical modifications (reviewed in ref. 12). Some of these antioxidant enzymes which have been previously studied in the skin include superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GPx), glutathione reductase (GR), and glutathione S-transferase (GST). Superoxide dismutase catalyzes the degradation of the superoxide anion into hydrogen peroxide. Catalase and glutathione peroxidase reduce the damaging effects of H_2O_2 by converting it into water and molecular oxygen. Glutathione S-transferase detoxifies foreign compounds, exhibits peroxidase activity, and binds reactive metabolites (14, 15). Glutathione reductase catalyzes the NADPH-dependent reduction of oxidized glutathione (GSSG) to glutathione (GSH) (14). When the scavenging rates of these antioxidant defense mechanisms are exceeded by the rate of the production of free radicals then cells are more inclined to suffer from oxidative damage (reviewed in ref. 11).

Since our laboratory did not see a direct correlation between UVB-induced inflammation and chronic UVB-induced tumor development between male and female mice, we hypothesized that the UVB-induced DNA damage and antioxidant capacities of each gender may not be equivalent. These published results demonstrated a significant difference in the percentage of 8-oxo-dG found in the cells of unirradiated and irradiated male and female skin. There were a greater percentage of 8-oxo-dG positive cells found in male mice before and after UV exposure compared to their female counterparts. In addition to determining the amount of oxidative damage present, the total antioxidant capacity was also assessed. Male mice had significantly lower antioxidant concentrations in both unirradiated and UVB exposed skin compared to the female mice. The

antioxidant activity in both male and female mice decreased after UV radiation compared to their no UV counterparts but was consistently significantly greater in female mice compared to male mice. There appears to be a gender disparity in not only the susceptibility to oxidative damage but the oxidative repair mechanisms found in unirradiated and UVB exposed skin (9).

The previous study on gender differences performed in our laboratory has shown that the gender differences in skin cancer and tumor development may be greatly influenced by the extent of oxidative DNA damage and antioxidant capacities, potentially more so than by the inflammatory response (9). To test these observations, the current study was performed to examine the temporal and gender differences in inflammation and specific antioxidant enzymes, including catalase, glutathione peroxidase, glutathione S-transferase, and glutathione reductase, after a single exposure to UVB. There does appear to be significant temporal differences in the antioxidant capacities after irradiation for both genders as well as a significant difference between the sexes in their respective antioxidant capacities before and after irradiation.

Methods and Materials

Animal Treatments

Both male and female Skh-1 outbred mice (n=4) (Charles River Laboratories, Wilmington, MA) were housed in The Ohio State University vivarium in accordance to the requirements set forth by the American Association for Accreditation of Laboratory Animal Care. Prior to the initiation of any studies, procedures were approved by the appropriate Institutional Animal Care Utilization Committee. Male and female irradiated mice were dorsally exposed once to 2240 J/m² UVB with Phillips FS40UVB lamps (American Ultraviolet Company, Lebanon, IN) fitted with Kodacel filters (Eastman Kodak, Rochester, NY) to ensure the emission of UVB light (290-320nm). The UVB dose of 2240 J/m² was determined by a UVX radiometer (UVB Inc., Upland, CA). The mice were kept 4 animals per cage and there was no sign of dorsal wounds due to fighting amongst the animals.

Acute UVB studies were performed to measure and compare the gender differences in the cutaneous antioxidant enzyme activities. Irradiated male and female mice were exposed once to 2240 J/m² UVB and sacrificed 6, 24, or 48 hours after exposure (n=4 for each gender at each time point). Succeeding sacrifice of irradiated and nonirradiated male and female mice, dorsal skin fold thickness was measured to evaluate edema, 10 mm punch biopsies were taken to assess myeloperoxidase (MPO) levels, and the remaining dorsal skin was saved and snap frozen in liquid nitrogen for cutaneous antioxidant analysis.

Quantization of Tissue Myeloperoxidase Levels

During the inflammation process, activated neutrophils release myeloperoxidase (MPO), an enzyme that converts hydrogen peroxide to hypochlorous acid. When levels of cutaneous MPO are determined they can be used as a measurement of the infiltration and activation of neutrophils which is indicative of the cutaneous inflammatory response. The 10mm punch biopsies were homogenized in 1.25mL of 0.5% hexadecyltrimethylammonium bromide in 50 mM potassium phosphate buffer at pH 6.0 and 4°C. For three consecutive rounds the samples were sonicated, frozen in liquid nitrogen, and then thawed. After the last round, the samples were centrifuged for 10 min at 13,000rpm at 4°C and the supernatant layers were separated and transferred to new tubes for analysis. The sample (10 uL) and substrate (290 uL of 0.167 mg/mL o-dianisidine dihydrochloride and 0.0005% H₂O₂ in 50 mM potassium phosphate buffer with pH 6.0) were collected for assay in a 96-well plate to determine MPO activity with a spectrophotometer (Molecular Devices, Menlo Park, CA) at 450 nm for a 5 min period. The MPO data are expressed as mean units of MPO in which 1U of MPO activity is required to degrade 1 umol of peroxidase per minute at 25°C. A standard curve was used to calculate the amount of MPO activity in each sample.

Catalase Activity

A Catalase Activity Assay kit (Cayman Chemical, Ann Arbor, MI) was used to determine the catalase activity in the unirradiated and irradiated whole skin of male and female mice. The whole skin tissues were originally ground to powder using a mortar and pestle and then combined with cold homogenization buffer (50mM potassium phosphate, 1mM EDTA, pH 7.0) at a ratio of 5ul buffer per mg tissue. The samples were

homogenized and then sonicated 10 times 1 second for three rounds followed by centrifugation at 10,000xg for 15 min at 4°C. The supernatant materials were separated and transferred to new tubes and assayed according to the manufacturer's instructions. Formaldehyde is produced when the catalase in the samples reacts with methanol in the presence of hydrogen peroxide. The amount of formaldehyde produced is measured spectrophotometrically using Purpald as a chromagen. Finally a formaldehyde standard is provided to determine the activity found in all the samples.

Glutathione Peroxidase Activity

Glutathione Peroxidase (GPx) catalyzes the reduction of hydroperoxides by using reduced glutathione. The Glutathione Peroxidase Activity Assay kit (Cayman) was used according to the manufacturer's instructions to determine the GPx activity in the male and female unirradiated and irradiated mice. Using a mortar and pestle, tissues were ground to a powder and then combined with cold homogenization buffer (50mM Tris-HCl, 5mM EDTA, 1mM DTT, pH 7.5) at a ratio of 5ul buffer per mg tissue. The samples were homogenized first and then sonicated for 3 rounds of 10 times 1 second. After sonication the samples were centrifuged at 10,000xg for 15 min at 4°C. The supernatant materials were then assayed according to the manufacturer's instructions. In this assay, GPx oxidizes glutathione to form oxidized glutathione which is then reduced by glutathione reductase and NADPH. A decrease in absorbance at 340 nm is observed with the oxidation of NADPH. The GPx activity in the sample is directly proportional to the rate of change in absorbance.

Glutathione Reductase Activity

Glutathione Reductase (GR) is a flavoprotein that catalyzes the NADPH dependent reduction of oxidized glutathione (GSSG) to the reduced glutathione (GSH). A high GSH / GSSG ratio is essential for protection against oxidative stress and to the detoxification processes with GST and the peroxidase activities of GPx. The Glutathione Reductase Activity Assay kit (Cayman) was used according to the manufacturer's instructions to determine the GR activity in the male and female unirradiated and irradiated mice. Using a mortar and pestle, tissues were ground to a powder and then combined with a cold homogenization buffer (100mM potassium phosphate, 2mM EDTA, pH 7.0) at a ratio of 5ul buffer per mg tissue. The samples were homogenized first and then sonicated for 3 rounds of 10 times 1 second. Following sonication, the samples were centrifuged at 10,000xg for 15 min at 4°C. The supernatant materials were collected and assayed according to the manufacturer's instructions. A decrease in absorbance at 340 nm is observed with the oxidation of NADPH. The GR activity in the sample is directly proportional to the rate of decrease in absorbance.

Glutathione S-Transferase

Glutathione S-Transferase (GST) is an enzyme that plays a key role in cellular detoxification by conjugating toxicants to glutathione. The GST Activity Assay kit (Cayman) was used to determine the GST activity in male and female unirradiated and irradiated mice. Using a mortar and pestle, tissues were combined with cold homogenization buffer (100mM potassium phosphate, 2mM EDTA, pH 7.0) at a ratio of 5ul buffer per mg tissue. The samples were homogenized first and then sonicated for 3

rounds of 10 times 1 second. Following sonication the samples were centrifuged at 10,000xg for 15 min at 4°C. The supernatant materials were collected and assayed according to manufacturer's instructions. The GST activity is measured by the conjugation of 1-chloro-2,3-dinitrobenzene (CDNB) with reduced glutathione. This conjugation is accompanied by an increase in absorbance at 340 nm which is directly proportional to the GST activity in the sample.

Results

Gender differences in acute UVB induced inflammation

Male and Female mice were exposed once to 1 MED of UVB and then sacrificed at 6h, 24, and 48h after exposure for analysis. The edematous response to UV exposure was measured by determining the skin fold thickness in the male and female mice (Figure 1A). The acute inflammatory response of each gender was measured by determining the amount of myeloperoxidase (MPO) activity (Figure 1B). MPO is an enzyme released by activated neutrophils which infiltrate into the skin during the inflammatory response. The unirradiated male mice had a slightly higher skin fold thickness compared to their unirradiated female counterparts. Male mice displayed a significant increase in their skin fold thickness at 24h and 48h after exposure where as female mice showed a significant increase at 6h, 24h, and 48h after exposure. MPO activity was fairly equivalent between the genders with no UV exposure. Female mice had a significant increase in MPO activity at 6h, 24h, and 48h after irradiation and male mice experienced significant increases only at 48h after exposure. At 6h after irradiation there appears to be a significant difference between male and female MPO activity but not at 24h or 48h.

Catalase Activity

Catalase activity was measured in the unirradiated skin of male and female mice and at the various time points, 6h, 24h, and 48h after one exposure to 2240 J/m² UVB (Figure 2). The female mice had more catalase activity than the male mice in both unirradiated and irradiated skin. Both male and female mice displayed a significant decrease in catalase activity by 48h after UV exposure ($p=0.013$ and 0.018 respectively) compared to their unirradiated counterparts.

Glutathione Peroxidase Activity

Glutathione peroxidase activity was assessed in unirradiated, male and female dorsal skin and at the various time points after one exposure to 2240 J/m² UVB. Female mice displayed a slight increase in GPx activity at all time points after UV exposure and overall had more GPx activity than the male mice (Figure 3). There was a significant gender difference in GPx activity at 6h and 24h after irradiation (p=0.005 and 0.006 respectively). Male mice experienced the most significant decrease in GPx activity at 24h after exposure (p=0.019) compared to their unirradiated counterparts. GPx activity appeared to be returning toward baseline levels by 48h after UV exposure.

Glutathione S-Transferase

Glutathione S-Transferase activity was determined in male and female unirradiated dorsal skin and at various time points after acute UVB exposure (Figure 4). The female mice had slightly higher GST activities in both unirradiated and irradiated skin compared to their male counterparts. Ironically, the GST in male mice was significantly increased at 6h after UV exposure (p=0.044) and then significantly decreased by 48h after irradiation (p=0.033). The GST activity in the female mice was constant 6h after irradiation and was also significantly decreased by 48h (p=0.032).

Glutathione Reductase Activity (Figure 5)

Glutathione reductase (GR) activity was determined in the dorsal skin of unirradiated male and female mice and at various time points after one exposure to 2240 J/m² UVB (Figure 5). There does not appear to be any significant differences in GR activity in either gender after UV exposure. Male mice experienced the greatest decrease

in activity at 6h after irradiation and begin to recover GR activity by 24h and even more so at 48h. Female mice had displayed rather constant GR activity at all time points. There are also no significant gender differences in the GR activity.

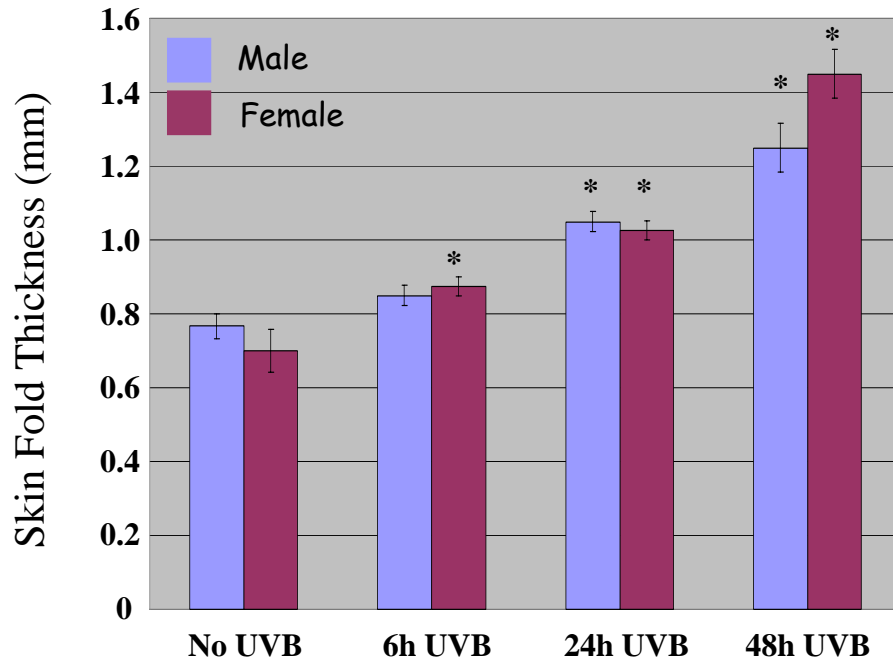
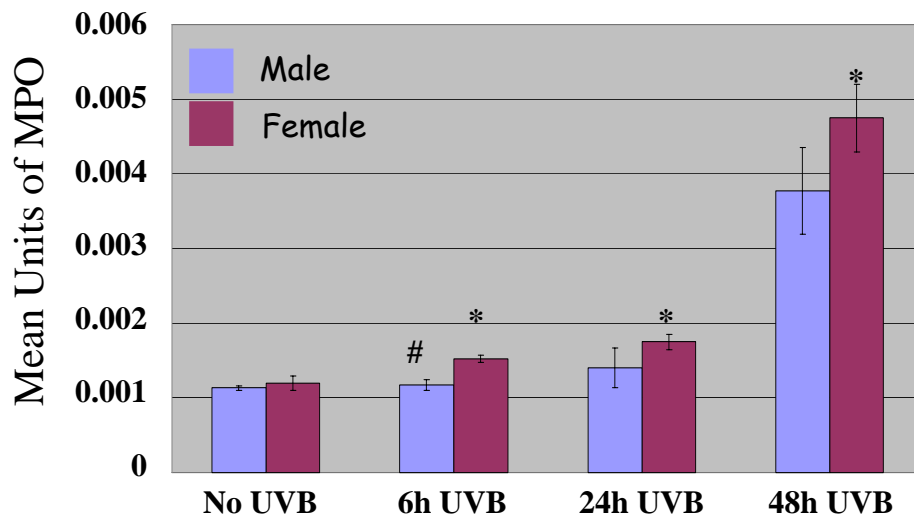
A**B**

Figure 1 Skin fold thickness (A) and MPO activity (B) were measured at the various time points after one exposure to 2240 J/m² UVB to evaluate and compare the edematous

response and the extent of inflammation between male and female mice. Both male and female mice had a significant increase in skin fold thickness at 24hr and 48hr compared to their unirradiated counterparts, however, the increase was significantly greater in females. The skin fold thickness between the genders was almost statistically significant ($p=0.071$) by 48hr after exposure. There was a significant difference in inflammation in the female skin after UV exposure at all time points. Males inherently had less of an inflammatory response to UV compared to females but had a significant increase in MPO levels by 48 hr compared to unirradiated skin. Columns, average; bars, \pm SE; *, difference to no UVB, $p<0.05$; # difference to females, $p<0.05$.

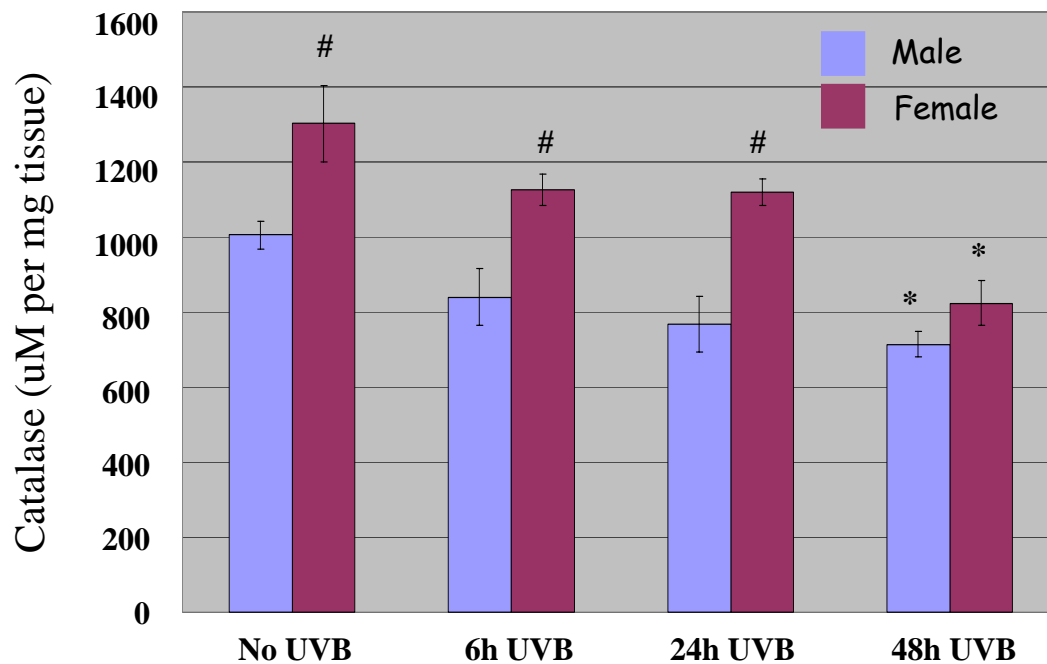


Figure 2 Catalase activity was measured in the skin of male and female mice at the various time points after one exposure to 2240 J/m² UVB. Both male and female mice had a maximal decrease in catalase activity at 48 hr after exposure. The male mice had less catalase activity compared to females in both unirradiated and irradiated skin. Columns, average; bars, \pm SE; *, difference to no UVB, $p < 0.05$; # difference to males, $p < 0.05$.

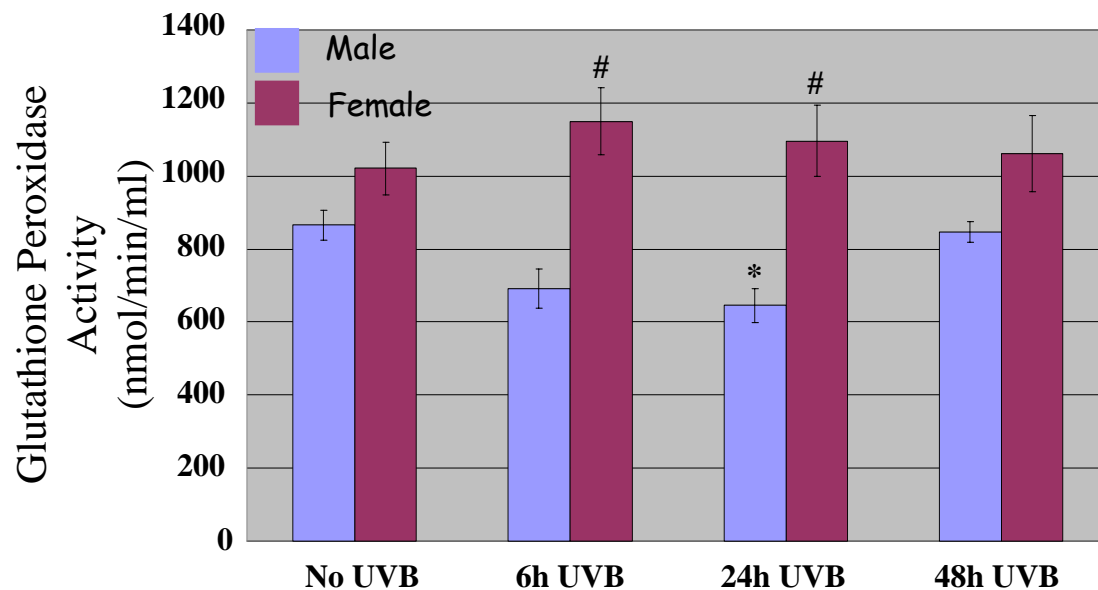


Figure 3 Glutathione Peroxidase activity was measured in the skin of male and female mice at the various time points after one exposure to 2240 J/m² UVB. Male mice had a significant decrease in activity by 24hr after exposure while the activity in females slightly increased and remained fairly constant. The activity in the male mice was significantly lower compared to the female mice at 6hr and 24hr after UVB exposure. Columns, average; bars, \pm SE; *, difference to no UVB, $p < 0.05$; # difference to males, $p < 0.05$.

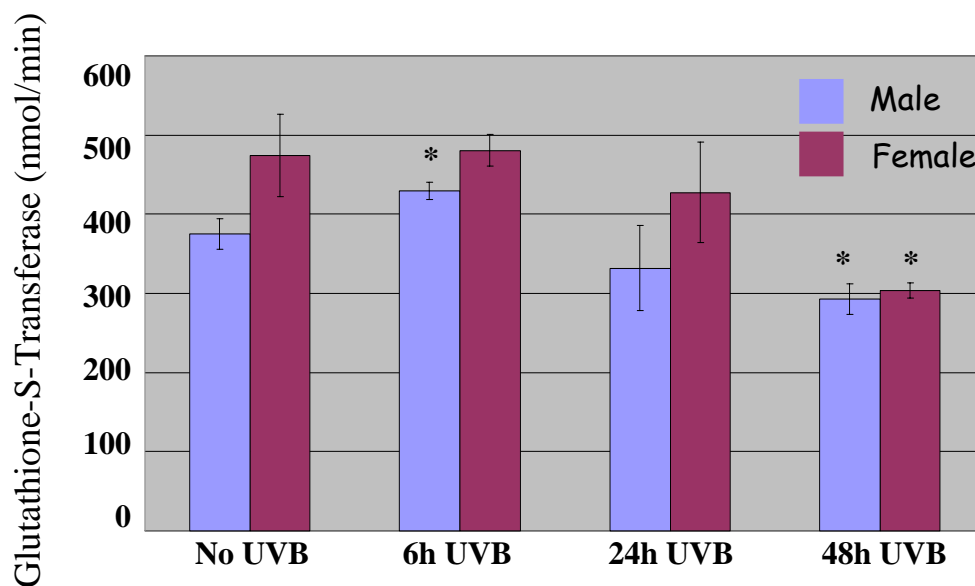


Figure 4 Glutathione S-Transferase activity was measured in the skin of male and female mice at the various time points after one exposure to 2240 J/m² UVB. Both male and female mice had a maximal decrease in Glutathione S-Transferase activity at 48hr after exposure. The activity in females was only slightly higher than the activity found in males in unirradiated and irradiated skin. Columns, average; bars, \pm SE; *, difference to no UVB, $p < 0.05$; # difference to males, $p < 0.05$.

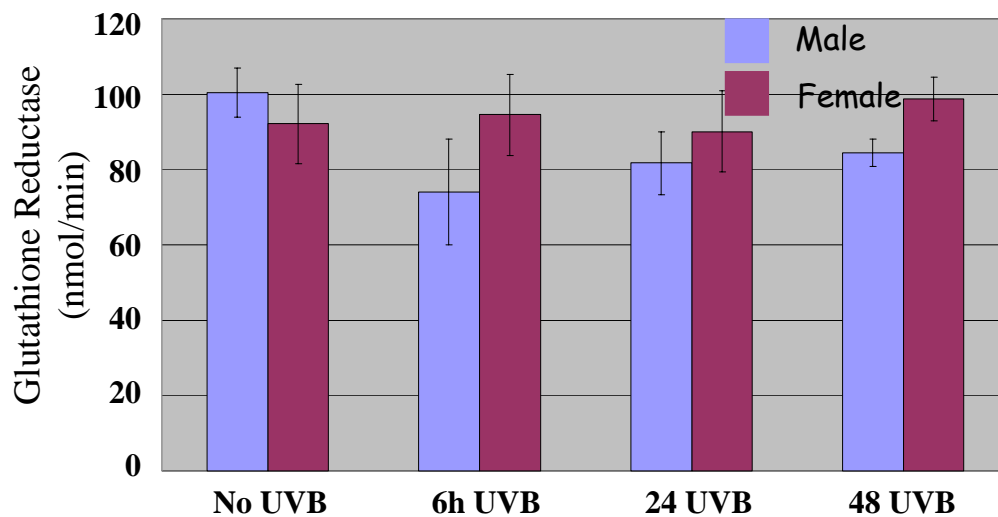


Figure 5 Glutathione Reductase activity was measured in the skin of male and female mice at the various time points after one exposure to 2240 J/m² UVB. There were no significant differences in Glutathione Reductase activity after UVB exposure and between male and female mice. The activity appears to decrease more in males by 48 hr compared to that of females (p=0.08). There is a trend of recovery of the GR activity for both genders by 48hr as well. Columns, average; bars, \pm SE.

Discussion

The current study used a well-established animal model to exhibit the temporal and gender differences in UVB-induced inflammation and specific antioxidant enzyme capacities. It has already been established through epidemiological and murine model studies that there is an apparent gender bias in the incidence of skin cancer and tumor development (1,2,3,9). Even though males tend to have more sun exposure and use less UV protection which may lead to a higher incidence of skin carcinogenesis (1,2,3), our laboratory has performed studies that suggest that the gender disparities may be the result of specific biological features rather than only sociologic variations (9). Inflammation is believed to play a role in driving the formation of tumors (5). This idea was applied to the skin and supported by the study performed in our laboratory in which the anti-inflammatory drug Celecoxib inhibited the acute inflammatory response after a single exposure to UVB and decreased the formation of tumors after chronic exposure to UVB in female mice (8). If murine studies parallel the epidemiological studies of skin carcinogenesis susceptibility, and inflammation does in fact contribute to tumor development, then it could be hypothesized that male mice might exhibit a greater degree of inflammation than female mice if exposed to equal doses of UVB. Our laboratory found, however, that when exposed to equal doses of radiation, male mice are more susceptible to tumor formation than female mice but have less of an inflammatory response (9). These results suggested that there was a biological difference in UV-induced tumor formation but it was not directly correlated with the inflammatory response (9).

The next step in the investigation was to compare the amount of oxidative damage present in the skin of each gender. Oxidative stress has previously been associated with the genesis of many diseases including rheumatoid arthritis, inflammation, photoaging, immunotoxicity, and skin cancer (14). Reactive oxygen species (ROS), produced by direct UV irradiation or indirectly through inflammatory cells, are the source for oxidative stress and the resulting mutations (16). Previous gender specific studies have found that the free radical production of 8-oxo-dG in hepatic mitochondrial DNA of male rats is 4-fold greater than their female counterparts (21). When the extent of oxidative DNA damage was determined by measuring the amount of 8-oxo-dG present in unirradiated and UVB exposed dorsal skin of male and female mice, it was discovered that males have more oxidative DNA damage in both unirradiated and irradiated skin compared to their female counterparts. This was contradictory to the notion that oxidative damage was partially caused by ROS produced by inflammatory cells because male mice had less of an inflammatory response but more oxidative damage than the female mice (9). This suggests that male mice may not be able to ward off the damaging effects of the ROS as well as female mice due to insufficient oxidative repair mechanisms. To verify this theory, the total antioxidant capacities of the unirradiated and UVB exposed dorsal skin of male and female mice were determined. The antioxidant concentrations in unirradiated and irradiated skin of male mice were significantly lower than that of their female counterparts. Both genders did, however, experience antioxidant depletion after UV radiation (9). There is an evident link between the increased oxidative DNA damage and decreased antioxidant activities found in the male mice.

The oxidative repair system is a biological entity that may be an influential factor in the gender variance found in the incidence of skin carcinogenesis. To follow up with the gender differences previously found in the total antioxidant concentrations in unirradiated and irradiated dorsal skin of male and female mice, the present study was designed to identify the gender differences in a select group of antioxidant enzymes. The select group chosen, which included catalase, glutathione peroxidase (GPx), glutathione s-transferase (GST), and glutathione reductase (GR), had already been previously studied in the skin (reviewed in ref.14, 15). There does appear to be inherent gender differences at baseline and after irradiation in the specific antioxidant enzyme capacities investigated in this study. In general, female mice appeared to have an increased antioxidant capacity compared to their male counterparts in both unirradiated and UVB exposed skin. There was a general trend of antioxidant depletion after UV exposure for the male mice while the female mice experienced some antioxidant depletion and some antioxidant continuity even after irradiation. Female mice had greater catalase activity than male mice at baseline and after UVB exposure. Catalase activity was maximally decreased by 48h after exposure in both genders. Other studies have also shown a decrease in catalase activity in murine and human skin models after UV exposure (17, 18) which parallel the results found for catalase in this study. Glutathione peroxidase decreased in the male mice after UV exposure and appeared to begin to recover by 48h. Female mice displayed an increase in GPx activity at 6h followed by a steady recover back to baseline levels by 48h. The female mice had greater GPx activity than the male mice at all time points measured. Other studies have found a decrease in the levels of GPx activity in the human skin after UV irradiation (19). Female mice had greater glutathione-s-transferase activity

than the male mice but these differences were not statistically significant. The GST activity appeared to maximally decrease by 48h after UV exposure in both genders. There were no significant temporal or gender differences in the glutathione reductase activity in the skin of the mice. The male mice had a slight decrease in GR activity at 6h but displayed recovery by 48h while the female mice showed a rather constant activity level at all time points.

The current study evaluated the antioxidant enzyme activities in whole dorsal skin of male and female mice at 6h, 24h, or 48h after one exposure to 2240 J/m² UVB. Additional evaluation of time points beyond 48h would determine if there are any further increases or decreases in the activities of the antioxidant enzymes after UVB exposure. The effect of UV exposure on the antioxidant enzymes may vary depending on the dosage of UVB radiation used. Some antioxidant enzymes may require more UVB exposure than others before initial depletion may occur. There may also be gender specific responses in the antioxidant capacities depending on the dosage of UVB. This study evaluated the antioxidant capacities in whole dorsal skin. Studies have been performed that demonstrate the antioxidant defense mechanisms in murine epidermis and dermis and their responses to UV irradiation (20). There have been no studies to date, however, demonstrating the gender differences in the antioxidant defense mechanisms in murine epidermis and dermis. Future studies are needed to determine the gender differences in the antioxidant defense mechanisms found in the skin after chronic exposure to UVB. This would provide insight into the antioxidant capacities present in different grades of UVB-induced tumors.

Several studies support the presence of other cellular components that are incorporated into the oxidative repair mechanisms of the skin in murine and human models, which we did not investigate in the current study. These cellular components include the enzymatic antioxidant superoxide dismutase (SOD) and the non-enzymatic antioxidants glutathione (GSH), vitamins, phytochemicals, and minerals, just to name a few (17, reviewed in refs. 11, 12,14). SOD catalyzes the reduction of superoxide anion to less reactive H_2O_2 and appears to play a role in tumor suppression and cellular differentiation in the skin (reviewed in ref. 14). GPx or CAT detoxifies the H_2O_2 produced during the SOD reaction which illustrates the intricate interactions of the antioxidant system as a whole (20, reviewed in ref. 14). Several studies have reported decreased SOD activity after exposure to UV radiation in the skin of both murine and human models (reviewed in ref. 14). Glutathione is a major component of the antioxidant system that can scavenge ROS through direct or indirect enzymatic reactions. GR catalyzes the reduction of oxidized glutathione (GSSG) to GSH. GPx and GST both use GSH as a co-factor in their enzymatic reactions (reviewed in ref. 14). With the molecular knowledge of GSH discussed, the insignificant temporal and gender differences found in the activity of GR in our study suggests that there are sufficient stores of GSH in the dorsal skin of mice for the function of GPx and GST. Additional studies would be needed to confirm the sufficient stores of GSH present in the unirradiated and UVB exposed dorsal skin of mice.

It has been shown that vitamin E delays or reduces UV-induced skin carcinogenesis in mice (reviewed in ref. 6). *in vitro* treatment of mouse keratinocytes with ascorbic acid significantly decreases the amount of detectable UVB-induced 8-

OHdG (reviewed in ref. 12), and oral and topical application of polyphenols before UVB irradiation protects mice from UV-induced skin cancer development (reviewed in ref. 13). Stable mineral levels are important in the prevention of cell injury which leads to oxidative stress and for proper antioxidant enzyme function (reviewed in ref. 11). These are just a few examples of studies conducted that demonstrate the protective roles of minerals, vitamins, phytochemicals, and other antioxidants in the defense against oxidative stress and the development of carcinogenesis (reviewed in refs. 6,11,12,13). There may be inherent gender differences in several of these cellular components involved in the defense mechanisms and not only in the specific antioxidant enzymes investigated in the present study. It is important to further investigate the specific characteristics, interactions, and functions of these cellular components to gain a better understanding of their potential roles in the development of skin carcinogenesis.

Gender differences have been reported in the development of several diseases (9). Prevention and treatment practices should be gender specific for the diseases that appear to have gender specific development. We can develop successful prevention and treatment plans for each gender specific disease or carcinogenesis, with an understanding of the biological components that vary between males and females. This study is a work in progress to find the biological gender differences that lead to the gender disparities found in the incidence of skin carcinogenesis. The results suggest that the gender differences in the inherent antioxidant defense mechanisms may in part contribute to the gender differences in the process of carcinogenesis. It has been theorized that oral intake of antioxidant vitamins and minerals may help prevent damage that results from UV exposure. Clinical trials, however, have revealed contradictory results. A study

performed by Hercberg et. al. (22) in which men and women were randomized to take a capsule containing a combination of antioxidants or a matching placebo for 7.5 y showed that the effect of antioxidant supplementation on the incidence of skin cancer varied according to gender. Women who received the antioxidant supplementation had a higher incidence of skin cancer than their placebo group while men did not display a difference in skin cancer development between the groups (22). In combination with the results of the present study, the Hercberg et. al. study suggests that the higher level of antioxidants that females display at baseline may overwhelm the defense mechanisms in the skin and lead to the development of skin cancer. This further solidifies the importance of preventing and treating cancer differently in each gender.

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